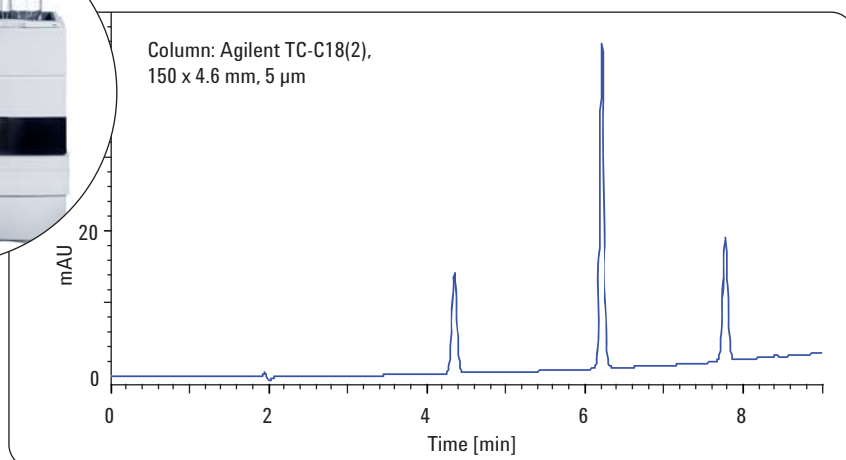
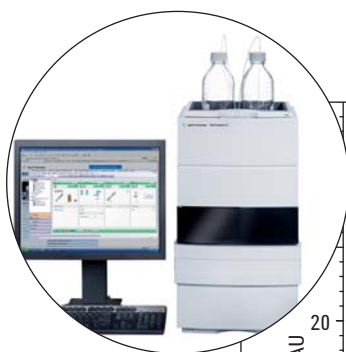


# In-process control for Aripiprazole manufacturing with the Agilent 1120 Compact LC and Agilent TC C18(2) columns

## Application Note

Siji Joseph,  
Patric Hörth



### **Abstract**

The Agilent 1120 Compact LC is the system of choice for conventional, analytical-scale liquid chromatography. It is an integrated LC designed for ease of use, performance, and reliability. It is well-suited for in-process control during drug manufacturing, due to the highly precise retention times and peak areas. This Application Note shows:

- Excellent retention time precision, with a relative standard deviation (RSD) < 0.07 %.
- Excellent area precision, with RSD < 0.25 % for baseline-separated peaks.

#### **Agilent Equipment**

- Agilent 1120 Compact LC
- Agilent TC C18(2) column

#### **Application Area**

- Pharmaceutical industry:  
QA/QC, drug manufacturing



**Agilent Technologies**

## Introduction

During manufacturing of Active Pharmaceutical Ingredients (APIs) it is mandatory to monitor the levels of precursors or by-products that are formed during the chemical reactions. To ensure these criteria, manufacturing chemists have to carry out several analyses called in-process analyses. HPLC is commonly used as a technique, primarily because it separates individual analytes and also helps to perform qualitative and quantitative analysis. The developed chromatographic methods must be robust and reliable over a number of years.

The purpose of the present study was to develop an HPLC method for the analysis of Aripiprazole and its precursors with desired specificity and sensitivity. This Application Note describes the advantages obtained from in-process control of Aripiprazole by reversed-phase high-performance liquid chromatography (RP-HPLC). The power to carry out thorough in-process control of samples taken during the course of the entire process is highlighted.

## Experimental

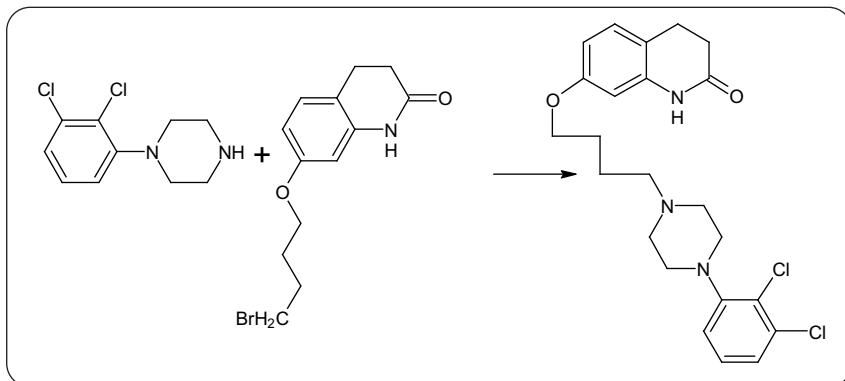
### Equipment

The Agilent 1120 Compact LC system included:

- A gradient pump with low-pressure mixing
- An autosampler with vial tray
- A column compartment for a column up to 250 mm in length
- A variable wavelength detector (VWD)



**Figure 1**  
Agilent 1120 Compact LC.



**Figure 2**  
The synthesis of Aripiprazole from starting materials 1 and 2.

The column was an Agilent TC (Typical Carbon load) C18(2) column, 150 x 4.6 mm, with 5  $\mu$ m particle size. The instrument was controlled by Agilent EZChrom Elite Compact Compliance software.

### Synthetic reaction

The antipsychotic drug Aripiprazole and its two starting materials were used for the study. 1-(2,3-Dichlorophenyl)piperazine hydrochloride (starting material-1) and 7-(4-bromobutoxy)-3,4-dihy-

drocarbostyryl (starting material-2) are the two starting materials for the synthesis of Aripiprazole. The synthetic scheme is shown in figure 2.

### Samples

In order to simulate in-process control samples, we prepared samples with varying concentrations of starting materials 1, 2 and the product Aripiprazole. During the reaction, the concentrations of the starting materials decrease, while the concentration of the product increases.

### Chromatographic parameters

Experiments were performed to establish the best chromatographic conditions for the analysis of Aripiprazole and related compounds. Based on these experiments, the following conditions were chosen:

- Sample: 1-(2,3-dichlorophenyl)piperazine hydrochloride, 7-(4-bromobutoxy)-3,4-dihydrocarbostyryl and Aripiprazole
- Column: Agilent TC-C18(2), 150 x 4.6 mm, 5  $\mu$ m
- Mobile phase:  
A = water + 0.2 % trifluoroacetic acid (TFA),  
B = acetonitrile + 0.16 % TFA  
(The TFA improves retention and peak shape.)
- Flow rate: 1.0 mL/min
- Gradient: at 0 min 30 %B, at 7 min 70 %B, then hold the ratio for two more minutes
- Injection volume: 5  $\mu$ L
- Autosampler programmed with a wash vial (acetonitrile) for rinsing exterior of the needle
- Run time: 9 min
- Post time: 5 min
- VWD: 254 nm, peak width (PW) > 0.05 min

### Sequence table

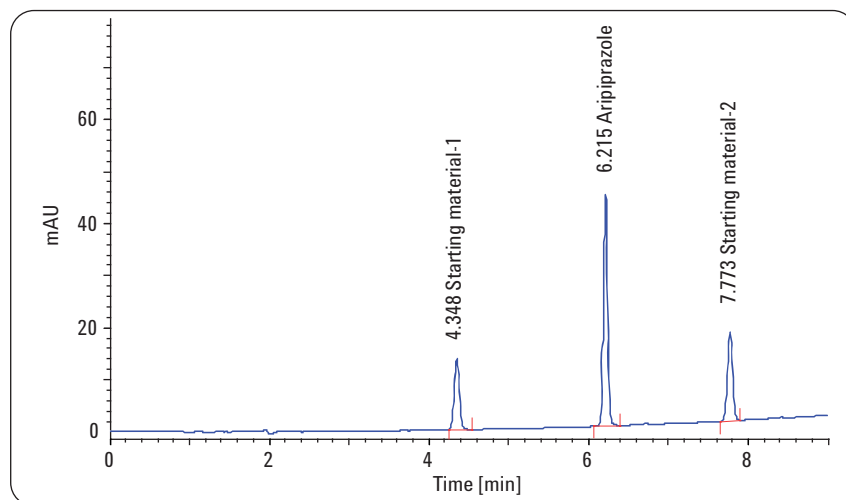
Table 1 shows the sequence table that was created. The concentrations of the three compounds were varied from vial 2 to vial 9, and each sample was injected three times so that an RSD could be calculated.

### Results and discussion

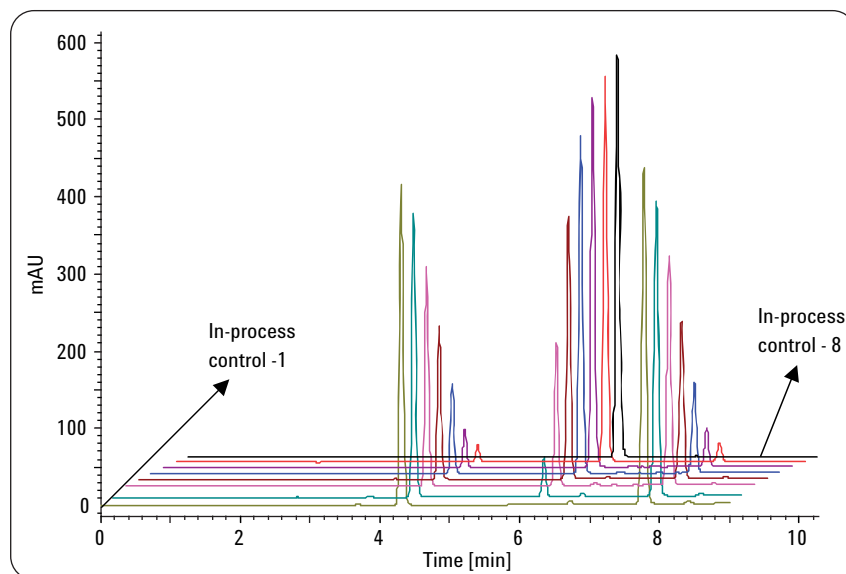
The chromatographic method was set up such that all compounds were baseline-separated. Figure 3 shows the separation of starting materials 1 and 2 and the product

Line	Location	Sample name	# Injections	Injection volume ( $\mu$ L)
1	Vial 1	Blank	2	5
2	Vial 2	In-process control-1	3	5
3	Vial 3	In-process control-2	3	5
4	Vial 4	In-process control-3	3	5
5	Vial 5	In-process control-4	3	5
6	Vial 6	In-process control-5	3	5
7	Vial 7	In-process control-6	3	5
8	Vial 8	In-process control-7	3	5
9	Vial 9	In-process control-8	3	5

**Table 1**  
Sequence table for the analysis.



**Figure 3**  
Chromatogram of Aripiprazole and starting materials 1 and 2, with retention times.



**Figure 4**  
Chromatographic overlay of the in-process control samples, (x-axis offset).

Aripiprazole. The separation time was nine minutes; the total run time (including equilibration) could be limited to 14 minutes, including re-equilibration time.

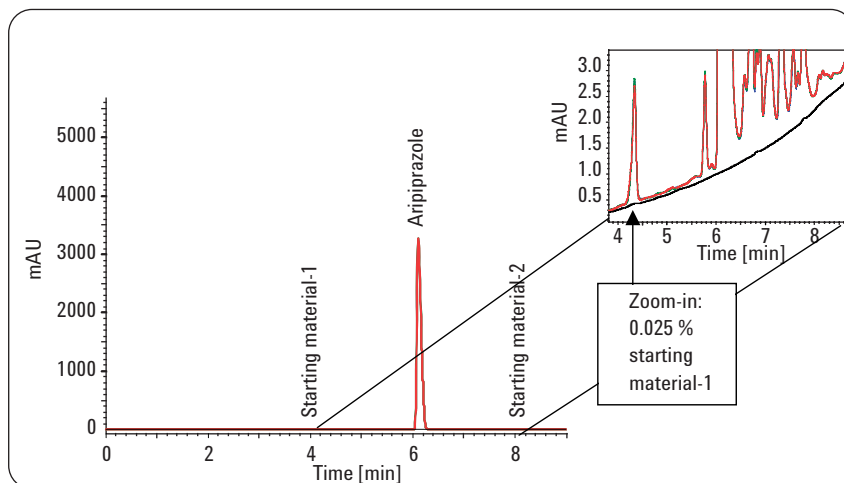
The chromatographic overlay of the reaction monitoring analysis is displayed in figure 4.

Figure 5 represents the overlay of the three injections of the last in-process control sample of this series, plus a blank run. The in-process control sample contained Aripiprazole and starting material-1 at a level of 0.025 % of the main compound. The zoom-in of the starting material area is shown.

The assay precision (RSD) was assessed by expressing the standard deviation of repeated measurements as a percentage of the mean value. Intra-day precision was estimated from three replicates at eight levels of a standard mixture. The retention time and area precisions of three consecutive injections of each in-process check sample are summarized in table 2. The RSD for peak area was less than 0.25 %.

## Conclusion

An HPLC method was developed for the simultaneous determination of Aripiprazole and potential precursor products. Furthermore, the chromatographic method was demonstrated to be useful for the in-process control of Aripiprazole



**Figure 5**  
Zoomed chromatogram of 0.025 % starting material-1 with respect to main compound.

Sample	RSD of retention time			RSD of area		
	Starting material 1	Aripiprazole	Starting material 2	Starting material 1	Aripiprazole	Starting material 2
1	0.059 %	-----	0.012 %	0.144 %	No peak	0.096 %
2	0.022 %	0.016 %	0.012 %	0.084 %	0.086 %	0.077 %
3	0.022 %	0.027 %	0.012 %	0.184 %	0.209 %	0.181 %
4	0.022 %	0.016 %	0.012 %	0.169 %	0.207 %	0.177 %
5	0.039 %	0.016 %	0.012 %	0.198 %	0.228 %	0.212 %
6	0.022 %	0.016 %	0.000 %	0.186 %	0.134 %	0.192 %
7	0.022 %	0.016 %	0.012 %	0.122 %	0.102 %	0.121 %
8	0.028 %	0.016 %	0.012 %	0.222 %	0.122 %	0.212 %

**Table 2**  
Summary of the in-process analysis results.

synthesis. The Agilent 1120 Compact LC system is an excellent choice for in-process control, as it provides reproducible results with high precision for retention times and areas. For our example, the precision for retention times was < 0.06 % RSD and for areas of baseline-separated peaks was < 0.23 % RSD.

*Siji Joseph is Application Scientist at Agilent Technologies Life Science Center in Bangalore, India.*

*Patric Hörth is R&D Chemist at Agilent Technologies in Waldbronn, Germany.*

[www.agilent.com/chem/1120](http://www.agilent.com/chem/1120)

© 2008 Agilent Technologies Inc.

Published April 1, 2008  
Publication Number 5989-8330ENA



**Agilent Technologies**